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XPF with mutations in its conserved nuclease domain is defective in DNA repair but functions in TRF2-mediated telomere shortening

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ABSTRACT

TRF2, a telomere-binding protein, is a crucial player in telomere length maintenance. Overexpression of TRF2 results in telomere shortening in both normal primary fibroblasts and telomerase-positive cancer cells. TRF2 is found to be associated with XPF–ERCC1, a structure-specific endonuclease involved in nucleotide excision repair, crosslink repair and DNA recombination. XPF–ERCC1 is implicated in TRF2-dependent telomere loss in mouse keratinocytes, however, whether XPF–ERCC1 and its nuclease activity are required for TRF2-mediated telomere shortening in human cells is unknown. Here we report that TRF2-induced telomere shortening is abrogated in human cells deficient in XPF, demonstrating that XPF–ERCC1 is required for TRF2-promoted telomere shortening. To further understand the role of XPF in TRF2-dependent telomere shortening, we generated constructs containing either wild type XPF or mutant XPF proteins carrying amino acid substitutions in its conserved nuclease domain. We show that wild type XPF can complement XPF-deficient cells for repair of UV-induced DNA damage whereas the nuclease-inactive XPF proteins fail to do so, indicating that the nuclease activity of XPF is essential for nucleotide excision repair. In contrast, both wild type XPF and nuclease-inactive XPF proteins, when expressed in XPF-deficient cells, are able to rescue TRF2-mediated telomere shortening. Thus, our results suggest that the function of XPF in TRF2-mediated telomere shortening is conserved between mouse and human. Furthermore, our findings reveal an unanticipated nuclease-independent function of XPF in TRF2-mediated telomere shortening.

1. Introduction

Mammalian telomeres are composed of TTAGGG repeats and associated proteins that function to protect and maintain telomeric DNA. Telomeric DNA consists of double-stranded telomeric tracts and a 3′ G-rich single-strand protrusion. The latter is protected through its invasion into the duplex TTAGGG repeats, forming a higher order structure known as the t-loop [1]. The telomere-binding protein TRF2 [2,3], a component of the Shelterin complex specific to telomeres [3], is a crucial player in telomere protection as well as telomere length maintenance. Loss of TRF2 function in both human and mouse cells induces immediate degradation of 3′ G-rich single-strand overhangs, resulting in the formation of telomere end-to-end fusions [4–6]. In addition to its protective role, TRF2 also regulates telomere length maintenance. Overexpression of TRF2 results in accelerated telomere shortening in human primary fibroblasts [7]. Excess TRF2 also promotes

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telomere shortening in telomerase-positive cancer cells [8]. It has been suggested that excess TRF2 enhances nucleolytic processing of telomere ends by recruiting a nuclease [7].

Several lines of evidence indicate that XPF–ERCC1, a structure-specific endonuclease, is a potential candidate for this unknown nuclease. Firstly, XPF–ERCC1 interacts with TRF2 at telomeres [9]. Secondly, the XPF–ERCC1 complex is required for degrading 3′ G-rich overhangs upon TRF2 inhibition [9]. Thirdly, Blasco and her co-workers have shown that mouse keratinocytes overexpressing TRF2 display telomere loss, which is dependent upon XPF [10]. However whether the actual nuclease activity of XPF–ERCC1 is needed for TRF2-induced telomere shortening remains unknown.

The XPF–ERCC1 complex functions as a structure-specific endonuclease. The complex cleaves on the 5′ side of bubble structures containing damaged DNA [11–13]. XPF–ERCC1 also cuts DNA duplexes adjacent to a 3′ single-stranded DNA flap [14]. Patients with hypomorphic mutations in XPF show repair disorder xeroderma pigmentosum (XP), a rare autosomal recessive disease characterized by severe sun sensitivity and high incidence of skin cancer [15]. XP is associated with defects in nucleotide excision repair (NER) and contains the seven complementation groups of XPs (XP-A–XP-G), representing genes whose products are involved in the different steps of NER [13]. Compared to other XP groups, all XP-F patients show mild sensitivity to UV and have late onset of skin cancer [16–18]. Although mutations in ERCC1 are not found in the human population, mice lacking ERCC1 function are also UV-sensitive [19,20]. Interestingly, XPF and ERCC1 null mice display other phenotypes that are not seen in mice with a defect in other NER components, suggesting additional functions for XPF–ERCC1 [19–24]. In agreement, the XPF–ERCC1 complex plays a role in crosslink repair and homologous recombination [25]. XPF–ERCC1 also has a role in maintaining genome integrity. The complex is involved in suppressing the formation of telomeric-DNA-containing double minute chromosomes [9].

The two components of the XPF–ERCC1 complex are co-dependent. Cells lacking XPF have reduced levels of ERCC1 and vice versa [16,26–30]. For the enzymatic action of XPF–ERCC1, the heterodimer is essential. While the presence of ERCC1 in the heterodimer of XPF–ERCC1 is indispensable for its nuclease activity in NER, the actual nuclease domain is found in XPF [31]. XPF contains the conserved GDX_nERKX_3D signature motif that is involved in metal-dependent nuclease activity [31,32]. Mutagenesis and biochemical analysis revealed that heterodimers with mutations in conserved nuclease domain of XPF such as D704A, E714A and K716A display either no or residual enzymatic activity [31].

Here we report that the XPF–ERCC1 complex is required for TRF2-induced telomere shortening in human cells as previously shown for TRF2-dependent telomere loss in mouse cells [10], suggesting a conserved function of XPF–ERCC1 in TRF2-mediated telomere shortening between mouse and human. However the nuclease activity of the XPF–ERCC1 complex essential for its role in repairing UV-induced DNA damage is dispensable for TRF2-promoted telomere shortening. Thus, our data reveal an unanticipated function of XPF–ERCC1 in TRF2-dependent telomere shortening that is distinct from its role in NER.

2. Materials and methods

2.1. XPF constructs, cell culture and retro viral infection

The cDNA for the wild type XPF gene was a kind gift from Jan Hoeijmakers. The wild type XPF gene was cloned into the pLPC retroviral vector (generously provided by Titia de Lange). XPF mutants D704A and E714AK716A were made from the expression construct pLPC-XPF using the QuickChange site-directed mutagenesis kit (Stratagene). Wild type XPF and mutant-XPF-containing constructs were retrovirally delivered into GM08437B (SV40-transformed XP2YO from Coriell) cells as previously described [9].

Cells were grown in DMEM medium with 10% fetal calf serum for Phoenix cells and GM08437B (Coriell) or 15% FCS for hTERT-BJ-LT (a gift from Titia de Lange) and XP51RO-hTERT supplemented with non-essential amino acids, glutamine, 100 U/ml penicillin and 0.1 mg/ml streptomycin. Retroviral gene delivery was carried out as previously described [7]. Phoenix amphotropic retroviral packaging cells were transfected with vector alone or the vector containing Myc-TRF2. At 36, 48 and 60 h post-transfection, the virus-containing medium was used to infect cells in the presence of polybrene (4 μg/ml). Twelve hours after the last infection, puromycin (2 μg/ml) or hygromycin (90 μg/ml) was added to the medium and the cells were maintained in the selection medium.

2.2. Adenovirus and UV irradiation of virus

The recombinant adenovirus, Ad5HCMVsp1*lacZ* [33] was obtained from Dr. Frank L. Graham, McMaster University. Ad5HCMVsp1*lacZ* carries the bacterial *lacZ* gene under the control of the human CMV-IE promoter (−299 to +72 relative to transcription start site) inserted into the E1 deleted region of adenovirus. The deletion in the El region of the genome renders the virus unable to replicate in most mammalian cells. Virus was propagated, collected and titred as previously described [34].

UV irradiation of the virus has been previously described [35]. Viral suspensions in 1.8 ml of unsupplemented medium were irradiated in 35 mm dishes on ice with continuous stirring using a General Electric germicidal lamp (Model G8T5) emitting predominantly at 254 nm at an incident fluoresence rate of 2 J/m^2/s (J-255 shortwave UV meter, Ultraviolet Products, San Gabriel, CA). Aliquots of 200 μl were removed for each exposure to the virus and diluted appropriately with unsupplemented medium.

2.3. Host cell reactivation (HCR) of the adenovirus encoded reporter gene

The use of a recombinant adenovirus to examine host cell reactivation of a UV-damaged reporter gene has been described previously [36]. Cells were seeded in growth medium at a density of 3.8×10^4 cells/well in 96-well microtitre plates. Between 10 and 24 h after seeding, media were aspirated from the microtitre plates and the cells were infected with either irradiated or unirradiated Ad5HCMVsp1 *lacZ* in a volume of 40 μl at a multiplicity of infection (MOI) of 20 plaque forming units (PFU)/cell. Following viral adsorption for 90 min at 37 °C, the cells were re-fed with warm growth medium.

2.4. Quantitation of β-galactosidase activity

Cells were harvested 40–44 h after infection. Infected cell monolayers were incubated with 1 mM chlorophenol red-β-D-galactopyranoside (CPRG; Boehringer-Mannheim, Indianapolis, IN) in 0.01% Triton X-100, 1 mM $MgCl_2$, and 100 mM phosphate buffer at pH 8.3. The absorbance at 570 nm was determined at several times following the addition of the β-gal substrate using a 96-well plate reader (Bio-Tek Instruments EL340 Bio Kinetics Reader).